

CHOO et al. - APPLICATION NO. 09/424,487

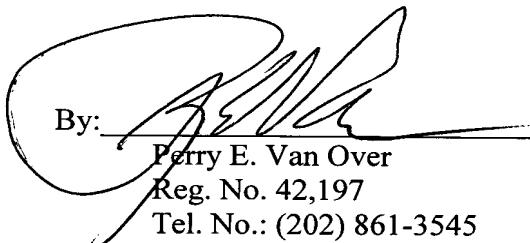
The Sequence Listing does no include new matter. A copy of the "Sequence Listing" in computer readable form is also submitted herewith, in accordance with 37 CFR 1.821(e) and includes no new matter as indicated in the attached Statement To Support Filing and Submission In Accordance With 37 C.F.R. §§ 1.821-1.825.

Furthermore, in accordance with 37 CFR 1.821(f), it is submitted that the contents of the paper copy and the computer readable copy of the Sequence Listing are the same.

In view of the above, it is respectfully submitted that the above-identified application complies with the Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures pursuant to 37 CFR 1.821-1.825.

Respectfully submitted,

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APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The specification is changed as follows:

Page 5, last full paragraph:

Figure 2 shows the amino acid sequence of three fingers (SEQ ID NOS: 12-14, respectively in order of appearance) used for phage display selection in the determination of recognition code.

Page 6, first full paragraph:

Figure 3 lists the sequence-specific zinc finger clones (SEQ ID NOS: 15-109, respectively in order of appearance) obtained from phage selections, and their binding site signatures.

Page 6, third paragraph:

Figure 5, illustrates the sequence-specific interactions selected for at position 2 of the α -helix, binding to position 1 of the quadruplet (SEQ ID NOS: 74, 73, 54, 15-16, 57-58, 70, 73, 75, 45, 67, 105, 84, 76, 77, 48, 38 41 and 65-66, respectively, in order of appearance).

Page 6, fourth paragraph:

Figure 6 illustrates the design of a zinc finger binding protein (SEQ ID NOS: 110-114, respectively, in order of appearance) specific for a G12V mutant ras oncogene;

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Page 10, line 20, fifth full paragraph:

Preferably, X₂₋₃ is G-K-A, G-K-C, G-K-S or G-K-G. However, departures from the preferred residues are possible, for example in the form of M-R-N (SEQ ID NO: 4) or (SEQ ID NO: 5) M-R.

Page 12, third paragraph:

Consensus zinc finger structures may be prepared by comparing the sequences of known zinc fingers, irrespective of whether their binding domain is known. Preferably, the consensus structure is selected from the group consisting of the consensus structure (SEQ ID NO: 6) P Y K C P E C G K S F S Q K S D L V K H Q R T H T G (SEQ ID NO: 5), and the consensus structure (SEQ ID NO: 7) P Y K C S E C G K A F S Q K S N L T R H Q R I H T G E K P (SEQ ID NO: 6).

Page 12, fourth paragraph:

The consensuses are derived from the consensus provided by Krizek et al., (1991) J. Am. Chem. Soc. 113:4518-4523 and from Jacobs, (1993) PhD thesis, University of Cambridge, UK. In both cases, the linker sequences described above for joining two zinc finger motifs together, namely TGEK (SEQ ID NO: 4) or TGEKP (SEQ ID NO: 5) can be formed on the ends of the consensus. Thus, a P may be removed where necessary, or, in the case of the consensus terminating T G, E K (P) can be added.

Page 13, third paragraph:

A “leader” peptide may be added to the N-terminal finger. Preferably, the leader peptide is (SEQ ID NO: 8) MAEEKP.

Page 35, second paragraph:

The first finger of the designer lead peptide is designed according to the rules set forth herein starting from a Zif268 finger 2 model to bind the quadruplet 5' -GCCG-3', which corresponds to 'anticodon' 10 of the designated binding site plus one 3' base. The finger has the following sequence [(SEQ ID NO: 10)] (SEQ ID NO: 9):

Page 35, fifth paragraph:

Given the similarity of the DNA subsites, the second and third fingers of the DNA-binding domain are direct repeats of this first finger, but in which the third α -helical residue which contacts base 3 of a quadruplet, +3, is mutated according to recognition rules, to histidine in finger 2 and asparagine in finger 3, such that the specificity of these fingers is predicted to be 5'-GGCG-3' (includes 'anticodon' 11) and 5'-GACG-3' (includes 'anticodon' 12) respectively. Thus the second and third finger polypeptides have the sequences [(SEQ ID NO:11)] SEQ ID NOS: 10 and 11, respectively)

Page 36, first paragraph, beginning at line 5:

A construct consisting of DNA sequences encoding the three fingers joined together, preceded by a leader MAEEKP (SEQ ID NO: 8) at the N-terminus, is cloned as a fusion to the minor coat protein (gene III) of bacteriophage Fd in the phage vector Fd-Tet-SN (Y. Choo, A. Klug, (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91, 11163-11167). In phage display screening K_d of 17nM, and to discriminate strongly against the wild-type sequence.

IN THE CLAIMS:

The claims are amended as follows:

14. (Amended) A method according to claim 13, wherein the model zinc finger is a consensus

zinc finger whose structure is selected from the group consisting of the consensus structure [(SEQ ID NO:5)] (SEQ ID NO: 6) P Y K C P E C G K S F S Q K S D L V K H Q R T H T G, and the consensus structure [(SEQ ID NO: 6)] (SEQ ID NO: 7) P Y K C S E C G K A F S Q K S N L T R H Q R I H T G E K P.

18. (Amended) A method according to claim 14, wherein the N-terminal zinc finger is preceded by a leader peptide having the sequence (SEQ ID NO: 8) MAEEKP.